Transcapillary Exchange Rates of Deuterium Oxide and Thiocyanate in the Forearm of Man[1, 2, 3]

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THE RATE AT WHICH DIFFUSIBLE, nongaseous substances transfer into and out of the circulation is not well understood. Disappearance slopes of injected labeled materials represent the integrated effect of filtration and reabsorption and therefore indicate the net rather than the absolute transfer of such substances out of the blood stream. Hence, in order to measure the absolute transcapillary movement of a diffusible substance it is essential that the rate of transfer of this material be determined in the early phases of the initial circulation prior to any significant return of filtered material from the interstitial fluid to the blood.

This can be accomplished by utilizing a technique of multiple sampling from the effluent vessel at intervals of seconds rather than minutes. In addition as pointed out by Pappenheimer and his associates (1) since the method depends on concentration measurements, the dilution of the labeled substance by the blood itself also must be known. This may be accomplished by mixing the labeled, diffusible material with a second nondiffusible substance prior to injection. Then the concentration of the nondiffusible substance in the recovered samples will indicate the dilution of both substances by the blood itself. A similar method has been devised by Chinard (2) and by Freinkel, Schreiner and Athens (3).

METHODS

The dye T-1824 (Evan's blue) was used as the nondiffusible tracer since it has the property of rapid mixing and binds to the relatively impermeable plasma proteins. Deuterium[4] oxide and sodium thiocyanate were the permeable tracer substances. Twenty per cent 'salt free' human serum albumin was added to this mixture in order to obtain a solution with an osmotic pressure within the physiologic range. The usual procedure was to mix, under sterile precautions, 5 ml. of deuterium oxide,

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[4] The monochromater used for determining the concentrations of deuterium was constructed by the Research Department of Leeds and Northrup Company, and was loaned on a field trial basis to the Heat and Power Division of the National Bureau of Standards.

0.5 ml. of 5 per cent T-1824, 1.0 ml. of 20 per cent sodium thiocyanate and 1.5 ml. of 20 per cent human serum albumin.

After withdrawing a sample of this mixture for analysis for T-1824, deuterium and thiocyanate concentrations, the remainder was taken up in a syringe and injected at an even rate into the branchial artery over a 2- to 6-second period. The technique of injection and of sampling which was similar to that described in another communication (4) involves injection into the brachial artery followed by rapid sampling from an antecubital vein. In all except one experiment the circulation to the hand was excluded by inflating a pneumatic cuff wrapped around the wrist to pressures well above the subject's systolic blood pressure.

The concentrations of T-1824 and thiocyanate were determined according to methods previously described (5). The concentration of deuterium oxide was determined by a new spectrophotometric method which is based on the emission spectra of atomic hydrogen and deuterium in a high frequency electrodeless discharge (6, 7). Using this method it is possible to measure concentrations of deuterium as low as 1 part in 6500 with an accuracy of ± 5 per cent with correspondingly greater accuracy at the higher concentrations measured in these experiments.

Typical T-1824 transit curves through the vessels of the forearm have been described previously (4). Following the appearance of the dye there is a rapid rise to peak concentrations followed by a more gradual decline. The concentrations of deuterium oxide and of thiocyanate were measured at several points along this curve, but particularly at the early period prior to or at peak concentrations of the dye.

The estimation of the percentage of the permeable substances which pass out of the blood is calculated from the formula:

$$L = \frac{C_x^t - C_x^m}{C_x^t} \times 100 \qquad (1)$$

where L is the percentage loss of the labeled substance (x) from the circulating blood, C_x^t is the concentration of x which would be present if none had left the circulation and C_x^m is the concentration of x experimentally determined in the sample. The calculation C_x^t is based on the premise that no significant amount of T-1824 is lost from the circulation in a single passage through the forearm capillaries. The concentration of T-1824, therefore, is an index of the volume of blood in which the injected materials have been diluted. In addition, the relative concentrations of dye and the permeable substances in the injected mixture are known. Correction must be made, however, for the passage of diffusible substances into the water of the red cells. In the present studies it has been assumed that complete equilibrium has occurred between the water of the red cells and the water of the plasma in each sample.

The above factors are accounted for as follows: consider a venous blood sample of volume V. This volume represents the sum of two contributing volumes, w the volume contribution due to the injected solution and y the volume contribution of the blood. If C_e is the concentration of T-1824 in the injecta, the concentration in the sample under consideration, C_e^m is given by

$$C_e^m = \frac{wC_e}{w + pyW_p} \qquad (2)$$

where p is the plasma fraction of the sample and Wp is the water fraction of plasma. Thus, the denominator is the *total* fluid volume available to the T-1824, while the

numerator is the *amount* of T-1824 in this volume. If there were no loss of permeable tracer substance from the blood, that is no diffusion, the expected concentration, C_x^t, would be

$$C_x^t = \frac{wC_x}{w + pyW_p + cyW_c} \qquad (3)$$

where C_x is the concentration of the labeled substance x in the injected mixture, c is the cell fraction (by volume) of the sample and W_c is the water fraction of cells. The denominator, therefore, is the *total* fluid volume in the blood available to the tracer substance including the fluid volume of the red cells.

Solving *equations 2* and *3* the following expression is found for C_x^t

$$C_x^t = \frac{C_x C_m^e}{C_e + \left[\frac{cWc}{pW_p}(C_e - C_e^m\right]} \qquad (4)$$

Of these quantities of the right hand side of the equation, C_x, C_e^m, C_e, c and p are measured for each experiment, while W_c and W_p are considered as constants of blood $\left(\frac{W_c}{W_p} = \frac{.65}{.90} = .72\right)$. After determining C_x^m the percentage loss (L) of the material can be calculated directly from *equation 1*.

Results

The transcapillary exchange of deuterium oxide was determined in the forearm vascular bed in four normal subjects and two hypertensive patients (table 1 and fig. 1). The mean value for the maximum observed loss of deuterium oxide from the circulation in the six subjects was 94 per cent, S.D. 2.6. No significant differences were observed between the normal and the hypertensive subjects.

The permeability of thiocyanate was determined simultaneously in five of the six subjects. The maximum observed loss of thiocyanate from the circulation was much less than that of deuterium oxide, the mean value for thiocyanate being 48 per cent, S.D. 7.4.

Since it seemed possible that the high local concentrations of thiocyanate used in these studies might alter normal capillary permeability, sodium chloride in physiologic concentration was substituted for thiocyanate in one experiment (*subject J. J.*, table 1). In this experiment the rate of loss of deuterium oxide did not differ significantly from the others in which thiocyanate was injected.

The maximum percentage loss of both deuterium oxide and thiocyanate from the circulation occurred early in the circulation of T-1824. At this time there also was a considerable decline in hematocrit values. In later samples the apparent loss of both substances diminished probably because of some return of filtered material to the circulation. Figure 1 illustrates a typical experiment in which the percentage loss of deuterium oxide decreased from 95 to 79.7 per cent and of thiocyanate from 55.2 to 20.8 per cent. The hematocrit fell from a control value of 39 to 32 at the peak of the dye concentration curve but returned gradually to 37 in the final samples.

Because of the possibility that such marked and rapid shifts in hematocrit might influence the exchange rates, the circulation to the hand was not excluded in *subject C. G.* (table 1), and the volume of injected materials was made as small as could be permitted without sacrificing accuracy of determination. In this subject

the hematocrit did not fall at any time during the collection period; yet the percentage losses of deuterium oxide and thiocyanate were, if anything, slightly higher than those found in the other experiments.

TABLE 1. PERCENTAGE LOSS OF DEUTERIUM OXIDE AND THIOCYANATE ION FROM THE CAPILLARIES OF THE FOREARM IN NORMAL AND HYPERTENSIVE SUBJECTS. T-1824 CONCENTRATIONS AND HEMATOCRIT VALUES ALSO ARE GIVEN

SUBJECT AND DIAGNOSIS	TIME FROM INJ., SEC.	T-1824 CONC., IN SAMPLE, MG. %	ESTIMATED LOSS OF DEUTERIUM OXIDE, %	ESTIMATED LOSS OF THIOCYANATE, %	HEMATOCRIT
F. O.	0				39
Normal	6	3.0	95.0	47.6	36
	8	8.6	94.6	55.2	31
	12	10.2	92.6	34.8	31
	16	6.9	88.6	41.3	32
	22	5.0	85.1	40.3	34
	32	3.2	79.7	20.8	37
M. A.	0				41
Normal	12	2.5	87.3	56.0	38
	16	12.0	95.2	53.3	32
	28	5.8	84.2	29.6	36
	40	2.8	72.5	22.2	39
C. G.	0				50
Normal	14	2.7	95.3	41.0	50
	18	5.1	96.4	46.4	51
	22	4.2	96.8	47.1	50
	32	2.6	87.2	40.0	51
I. E.	0				43
Hypertension	14	1.6	86.7	34.8	42
	20	4.7	90.9	46.2	38
	30	3.6	88.6	49.1	42
	40	2.5	80.5	48.3	43
J. J.	0				42
Normal	14	3.5	95.8	*	38
	18	5.4	94.4		38
	24	3.4	90.0		39
	32	1.3	76.6		41
M. C.	0				37
Hypertension	10	6.2	91.6	34.1	32
	14	8.6	88.8	35.6	30
	16	8.4	90.5	37.8	30
	22	5.1	86.4	33.1	33
	34	1.9	65.5	16.9	35

* Sodium chloride replaced thiocyanate in this experiment.

DISCUSSION

The validity of the method depends primarily on the assumption that T-1824 is not lost from the blood plasma in any significant amounts during a single circulation. This assumption seems to be valid (8, 9). It is worth noting that if the capillaries were permeable to T-1824 the calculated loss of deuterium oxide would be less than the actual loss. Hence, it is not possible to ascribe the high rate of loss of deuterium oxide to leakage of T-1824.

Complete mixing of T-1824 with the circulating blood does not seem to be as important in this determination as in the measurement of total blood volume or

cardiac output. The dye is well mixed with the diffusible labeled substances prior to injection. Hence, if complete mixing of T-1824 with blood does not occur, the same should hold for the diffusible substances (except for differences in diffusion rates of the various substances).

It is possible that the deuterium may exchange with hydrogen atoms in the plasma proteins so that they are diluted into a larger volume than the plasma water. However, addition of deuterium oxide to plasma or blood *in vitro* indicated that errors due to such exchanges were negligible.

It is evident that the present method indicates only the loss of permeable substances from the circulating blood and does not differentiate between passage through

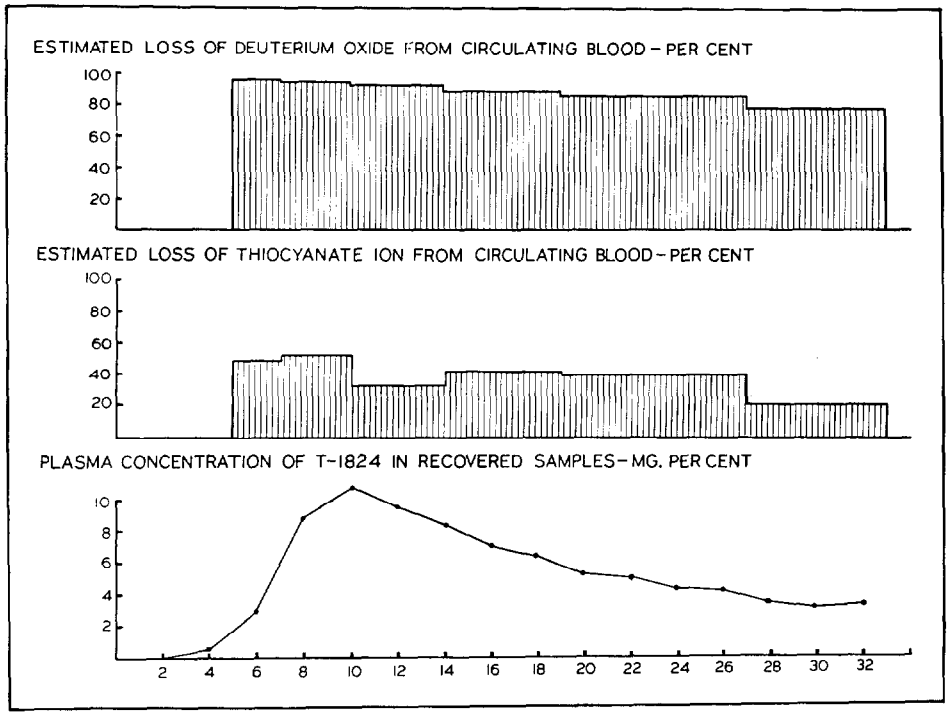

Fig. 1. PERCENTAGE LOSS of deuterium oxide and thiocyanate from the blood during a single circulation through the capillaries of the forearm. The T-1824 transit curve is shown below. *Ordinates* are percentages; *abscissae*, seconds.

the capillary membrane and penetration into the cells of the blood vessel walls. Finally, permeability may vary in vascular beds other than those in the forearm so that results obtained in this area of the circulation are not transferable to other areas.

The blood-extravascular fluid exchange was almost complete in a single circulation. It does not seem likely that hydrostatic pressure alone could accomplish such an extensive movement of fluid. Further, if water were lost in the arteriolar end of the capillary by hydrostatic pressure and regained in the venous end by osmotic pressure as has been supposed (10) the capillary hematocrit would of necessity rise to unphysiological heights. The magnitude of the water exchanges found in these experiments makes it seem possible that the transcapillary movement of water is

primarily a process of simple diffusion, whereas the forces of hydrostatic and osmotic pressure are more concerned with the adjustment of the final equilibrium. Flexner and his associates using a slightly different technique also concluded that diffusion rather than filtration is the predominant process in the exchange of water, sodium and chloride across the capillary wall (11).

It is of interest that the descending limb of the curve representing the percentage loss of deuterium oxide was not steep, indicating that the heavy water was not returning to the blood either rapidly or in large amounts. This suggests that the greater part of the filtered water immediately enters cells, or moves away in the interstitial fluid or becomes bound; and that the greater part of the water entering the blood in that period has not been filtered recently through the capillaries. Such observations suggest an extensive migration of water in the extravascular tissues.

The percentage loss of thiocyanate ion from the circulating blood was less than that of water. The lower diffusion rate of thiocyanate may be accounted for by the fact that significant amounts of thiocyanate bind to plasma proteins particularly in the albumin fraction (12) and, therefore, cannot penetrate the capillary wall. In addition the larger thiocyanate molecule does not diffuse as rapidly as deuterium oxide.

The descending limb of the curve representing the percentage loss of thiocyanate (fig. 1) usually was steeper than the similar curve for deuterium oxide indicating that thiocyanate returns more rapidly to the blood. This difference in reabsorption rates is not surprising, however, since thiocyanate does not penetrate most cells (13) and therefore remains in the interstitial fluid where it is available for more rapid equilibration with the circulating blood.

When the volume perfused was small in comparison to the volume injected the hematocrit fell indicating a considerable disturbance in this physiologic equilibrium of the blood. However, despite a massive water exchange this new equilibrium was maintained in the diluted segment of blood moving through the forearm capillaries. The fluid content of the blood, therefore, does not seem to be adjusted immediately by physical forces operating in capillary beds such as those in the forearm.

Summary and Conclusions

A method is described for estimating the exchange rates of labeled filterable substances through capillary walls. The method was applied in studying the transcapillary exchange rates of deuterium oxide and thiocyanate ion, and indicated that approximately 95 per cent of the heavy water and 50 per cent of the thiocyanate ion transferred out of the blood in a single circulation through the forearm. The major portion of the deuterium oxide which passed through the capillary walls of the forearm did not return rapidly to the circulation.

The rapidity and magnitude of the water exchange across capillary walls observed in these experiments suggest that simple diffusion rather than hydrostatic and osmotic pressures is the major influence governing such fluid transfer.

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REFERENCES

1. Pappenheimer, J. R., E. M. Renkin and L. M. Borrerd. *Am. J. Physiol.* 167: 13, 1951.
2. Chinard, F. P. *Bull. Johns Hopkins Hosp.* 88: 489, 1951.
3. Freinkel, N., G. E. Schreiner and J. W. Athens. *J. Clin. Investigation* 31: 629, 1952.

4. FREIS, E. D., J. R. STANTON AND C. P. EMERSON. *Am. J. Physiol.* 157: 152, 1949.
5. FREIS, E. D. AND R. H. SMITHWICK. *Am. J. M. Sc.* 214: 363, 1947.
6. BROIDA, H. P. AND G. H. MORGAN. *Analy. Chem.* 24: 799, 1952.
7. MOROWITZ, H. J. AND H. P. BROIDA. *Analy. Chemi.* In press.
8. GREGERSEN, M. I. AND R. A. RAWSON. *Am. J. Physiol.* 138: 698, 1943.
9. BARNES, D. W. H., J. F. LOUTIT AND E. B. REEVE. *Clin. Sc.* 7: 155, 1948.
10. BEST, C. J. AND N. B. TAYLOR. *The Physiologic Basis of Medical Practice.* Baltimore: Williams & Wilkins, 1945, p. 25.
11. FLEXNER, L. B., D. B. COWIE AND G. J. VOSBURGH. *Cold Spring Harbor Symposia on Quantitative Biology* 13: 88, 1948.
12. SCHEINBERG, I. H. AND H. J. KOWALSKI. *J. Clin. Investigation* 29: 475, 1950.
13. CRANDALL, L. A., JR. AND M. X. ANDERSON. *Am. J. Digest. Dis. & Nutrition* 1: 126, 134.